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Effect of Igran on the Rhizosphere Mycoflora of *Vicia faba* Plants Grown in Soils Infested with *Orabanche crenata* and Amended with *Rhizobium leguminosarum*

Ahmed M. Shalaby, Mohammed A. Rizk and Tarek A. A. Moussa
Department of Botany, Faculty of Science, Cairo University, Giza 12613, Egypt

Abstract: Either the recommended or double recommended igran dose significantly reduced the rhizosphere fungal, actinomycetal and bacterial count. The faba plants were unable to tolerate the 4-fold igran dose, thus enriching the rhizosphere with organic matter that helped flourishing the microorganisms in this area. The drop in soil invertase or amylase activity in igran-treated samples was far greater in the rhizosphere than the surrounding soil of the faba beans, whereas the differences in the cellulolytic activity was almost negligible. The biomass gain by the test fungi was hardly, if at all, affected by the presence of recommended igran dose a response that continued with larger dose application except *Aspergillus ochraceus* where it significantly stimulated the dry weight gain.

Key words: *Vicia faba*, rhizosphere, igran, enzyme activity, microbial count

Introduction

The rhizosphere region is biologically more active than the soil farther away. The increased activity in this region is believed to be due to the presence of greater microbial population, which, in turn, is influenced by the various biologically active chemicals exuded by the plants roots. Also the presence of the hydrolytic enzymes such as invertase and amylases, in greater concentrations can be attributed to the greater biodegradation activity in the rhizosphere region. The origin of these enzymes might be from root exudation and the exogenous enzymes produced by the rhizosphere microorganisms (Balasubramanian *et al.*, 1970).

The influence of herbicides on legume species growth, nodulation and control of weeds have been reported. Some herbicides not only affect plant growth (Osborne and Robson, 1993) but also the nodule activity (Eberbach and Douglas, 1991). Al-Wakeel and Shalaby (1994) emphasized igran in counteracting the harmful effects of *Orabanche crenata* on *Rhizobium*-inoculated faba plants. Pre-emergence application of the herbicides recommended dose improved the growth of infected plants, causing adverse effects on nodulation and nitrogenase activity compared with the untreated host. El-Abyad *et al.* (1983) and Sawicka *et al.* (1998) reported that herbicides might be stimulatory, inhibitory or toxic to specific groups of microorganisms. Rennie *et al.* (1985) and Brazauskiene (1998) reported the effect of fungicides on the fungal diseases and nitrogenase activity of faba bean.

This investigation was conducted to evaluate the effect of different concentrations of igran on the microorganism's population and enzymatic activity within the rhizosphere of faba plants and the surrounding soil.

Materials and Methods

Faba bean (*Vicia faba* L. cv. Giza 3) seeds were sown in 30-cm diameter porous pots containing 10kg clay-loam soil (pH 7.5). Igran [methylthiotriazine] (80% active material) was applied one day before planting at the rate of 1, 2 and 4 times the recommended dose (2.89kg ha^{-1}) as water suspension onto the soil top, stirred to insure that it was well incorporated.

The plants were maintained in a green house under natural conditions and watered to field capacity throughout the experimental duration. After emergence, the plants were thinned to 4 uniform seedlings per pot.

Sixty days post sowing, samples (4 replicates), collected from the rhizosphere and surrounding soil of *Vicia faba* plants were assayed for invertase, amylase and cellulase activity according to Balasubramanian *et al.* (1970). The population of bacteria, actinomycetes and fungi, in the rhizosphere and surrounding soil, was estimated using soil extract agar, starch casein agar and Czapek agar medium respectively (Johnson and Curl, 1972), following the dilution plating technique. Warcup's soil plate method (Warcup, 1950) was also applied to calculate the percentage of occurrence of each fungous species.

Liquid Czapek-Dox medium containing 0, 5, 10, 15 or 45 ppm igran (μ/ml) was the culture medium for the growth of some representative fungi (3 replicates). Dry weight of 10-days old mats, incubated at 25°C was taken as a criterion for growth.

Statistical analysis was carried out according to Snedecor and Cochran (1980), using LSD to compare the significance of the results.

Results

R/S value: Either the recommended or the double recommended igran dose significantly reduced the rhizosphere fungal count, almost to the same extent, whereas the drop in the bacterial count was slightly alleviated by increasing the herbicide dose (Table 1). However, doubling the recommended igran dose alleviated the drop in the actinomycetal count. Four-folds herbicide level very highly significantly increased the fungal and actinomycetes counts and completely alleviated the inhibitory effects on the bacterial count. The same trends apply to the surrounding soil micro flora with minor difference. All the R/S ratios were increased at the recommended dose and decreased in other doses.

Fungal spectrum: The experimental soil contained 22 fungal species belonging to 12 genera of which *Aspergillus* were highly represented (5 spp.) followed by *Fusarium* and *Penicillium* (3 spp. each), *Helminthosporium* and *Trichoderma* (2 spp. each), then 7 genera represented by one species each (Table 2).

Of the rhizosphere mycoflora, *Aspergillus clavatus, A. flavus, A. fumigatus, Cladosporium herbarum, Cunninghamella echinulata, Helminthosporium sativum, Mucor racemosus, Penicillium chrysogenum, Trichoderma hamatum,* and *T. viride* were very sensitive to all concentrations of igran. *Aspergillus ochraceus, Fusarium solani* and *Rhizopus nigricans* were very sensitive to the 4-fold igran concentration only. *Fusarium moniliforme, Helminthosporium microsorum, Penicillium lanosum* and *P. oxalicum* did not appear in the higher concentrations of igran even in the control samples. *Aspergillus carbonarius* did not appear in the recommended dose samples only and *Cephalosporium acremonium* was absent in the recommended and control samples. *Acremonium furcatum* appears only in the 4-fold igran concentration, while *Fusarium culmorum* appears in the 2-fold igran concentration only.

Of the surrounding mycoflora, *Acremonium furcatum, Cunninghamella echinulata, Fusarium solani, Mucor racemosus, Penicillium oxalicum, Rhizopus nigricans* and *Trichoderma viride* did not appear in the surrounding soils of all igran doses, even in the control samples. *Aspergillus clavatus, Cladosporum herbarum, Fusarium moniliforme* and *Helminthosporium microsorum* were disappeared in the higher igran concentrations. *Altemaria alternata,*

Table 1: Microorganisms prevalence and their R/S ratios in the rhizosphere and surrounding soil of *Vicia faba* plants grown in soils infested with *Orabanche crenata*, amended with *Rhizobium leguminosarum* and supplemented with igran at the recommended dose or its multiples

Treatments		Number/g air dry soil								
		Rhizosphere			Surrounding soil			R / S ratio		
		F (x 10^3)	A (x 10^3)	B (x 10^4)	F (x 10^3)	A (x 10^3)	B (x 10^4)	Fungi	Actinomycetes	Bacteria
Control		35.6	49.8	170.6	18.0	26.2	116.0	2.0	1.9	1.5
1 dose		23.0*	42.6**	129.0*	4.6	21.2*	71.0*	5.0	2.0	1.8
2 doses		24.0*	47.2	141.0*	10.3	25.7	130.0	2.3	1.8	1.1
4 doses		63.3**	54.3*	184.5	52.3**	32.4*	144.5	1.2	1.6	1.3
LSD	5%	10.3	4.2	29.5	12.0	3.7	30.8			
	1%	15.1	6.1	41.8	13.8	6.3	45.2			

F, fungi; A, actinomycetes; B, bacteria *,** Significant at 5 and 1% level respectively

Table 2: Fungal species and their prevalence value (%), isolated from the rhizosphere (R_1-R_4) and surrounding soil (S_1-S_4) of 60-days old *Vicia faba* plants grown in soil infested with *Orabanche crenata*, amended with *Rhizobium leguminosarum* and supplemented with igran at the recommended dose (10mg/pot) or its multiples. (+ , present; -, absent)

Species	Control		1 dose		2 doses		4 doses		Prevalence %	
	R_1	S_1	R_2	S_2	R_3	S_3	R_4	S_4	R	S
Acremonium furcatum (F. & V. Moreau) ex. Gams	-	-	-	-	-	-	+	-	25	0
Alternaria alternata (fr. Keissler)	+	-	+	-	-	+	-	-	50	25
Aspergillus carbonarius (Bainier) Thom	+	+	-	-	+	-	+	+	75	50
A. clavatus Desmazieres	-	+	-	+	-	-	-	-	0	50
A. flavus Link	+	-	-	-	-	+	-	-	25	25
A. fumigatus Fresen.	-	+	-	-	-	+	-	-	0	50
A. ochraceus Wilhelm	-	-	+	+	+	+	-	+	50	75
Cephalosporium acremonium Corda	-	-	-	-	+	-	+	-	50	0
Cladosporium herbarum (Persoon) Link	+	-	-	+	-	-	-	-	25	25
Cunninghamella echinulata Thaxter	+	+	-	-	-	-	-	-	25	25
Fusarium culmorum (W. G. Smith) Sacc.	+	-	-	-	+	+	-	-	50	25
F. moniliforme Sheld	-	-	+	+	-	-	-	-	25	25
F. solani (Mart.) Sacc.	-	-	+	-	+	-	-	-	50	0
Helminthosporium microsorum Sacc.	-	-	+	+	-	-	-	-	25	25
H. sativum Pammel, King & Bakke	-	-	-	+	-	+	-	-	0	50
Mucor racemosus Fresen.	+	+	-	-	-	-	-	-	25	25
Penicillium chrysogenum Thom.	+	+	-	-	-	-	-	+	25	50
P. lanosum Westling	-	+	+	-	-	-	-	+	25	50
P. oxalicum Thom.	-	-	+	-	-	-	-	-	25	0
Rhizopus nigricans Ehrenb. A7	-	-	+	-	+	-	-	-	50	0
Trichoderma hamatum (Bon.) Bain.	-	-	-	-	-	-	-	+	0	25
T. viride Pers, Fr.	+	-	-	-	-	-	-	-	25	0
Number of species	9	7	8	6	6	6	3	5		

Table 3: Fungal species (per 1g air dry soil x10^3) prevailing in the rhizosphere (R_1-R_4) and surrounding soil (S_1-S_4) of 60-day old *Vicia faba* plants grown in soil infested with *Orabanche crenata*, amended with *Rhizobium leguminosarum* and supplemented with igran at the recommended dose (10mg/pot) or its multiples (-, Fungus absent)

Species	Control		1 dose		2 doses		4 doses	
	R_1	S_1	R_2	S_2	R_3	S_3	R_4	S_4
Acremonium furcatum	-	-	-	-	-	-	10	-
Alternaria alternata	11	-	12	-	5	15	-	-
Aspergillus carbonarius	13	12	-	-	8	-	5	4
A. clavatus	-	10	-	5	-	-	-	-
A. flavus	6	-	-	-	-	4	-	-
A. fumigatus	-	13	-	-	-	10	-	-
A. ochraceus	14	12	12	-	13	-	19	22
Cephalosporium acremonium	-	-	-	-	4	-	2	-
Circinella simplex	-	5	-	-	-	-	-	-
Cladosporium herbarum	15	21	12	-	-	-	-	-
Cunninghamella echinulata	31	20	-	-	-	-	-	-
Fusarium culmorum	10	-	-	-	3	1	-	-
F. moniliforme	-	-	14	11	-	-	-	-
F. solani	-	-	16	-	3	-	-	-
Helminthosporium microsorum	-	-	12	5	-	-	-	-
H. sativum	-	-	-	3	-	1	-	-
Humicola brevis Gilman	-	-	-	2	-	2	-	-
Mucor racemosus	25	23	-	-	-	-	-	-
Mycelia sterilia	-	5	-	-	-	-	-	-
Penicillium chrysogenum	13	6	-	-	-	-	-	2
P. lanosum	10	8	4	-	-	-	-	-
P. oxalicum	-	-	7	-	-	-	-	-
Rhizopus nigricans	-	-	12	-	10	-	-	-
Stachybotrys atra Corda	-	-	-	-	9	-	-	-
Torula lanosa Szilv.	5	4	-	-	-	-	-	-
Trichoderma hamatum	-	-	-	-	-	-	20	5
T. viride	6	1	-	-	-	-	-	-
Number of species	12	13	9	5	8	6	5	4

Table 4: Effect of igran, at its recommended dose (10 mg/pot) or its multiples, on some hydrolytic enzymes activity (U g^{-1} air dry soil) in the rhizosphere (R_1-R_3) and surrounding soil (S_1-S_3) of 60-days old *Vicia faba* plants growing in soil infested with *Orabanche crenata* and amended with *Rhizobium leguminosarum*

Treatments	Invertase		Amylase		Cellulase	
	R_1	S_1	R_2	S_2	R_3	S_3
Control	280.7	109.2	124.2	64.4	152.0	146.3
1 dose	216.2	81.6	119.8	62.7	165.4	148.6
2 doses	266.0	92.0	104.5	69.6	123.2	149.9
4 doses	312.4	123.0	92.4	72.8	175.7	158.5
LSD 5%	31.3	13.5	18.9	8.2	12.1	11.8

Table 5: Biomass yield (mg) by selected fungi supplemented with different concentrations of igran in their Czapek-Dox medium after 12 days incubation at 25°C

Fungus	Concentration (μg/ml)				LSD	
	0	5	15	45	5%	1%
Aspergillus ochraceus	260.2	269.1	305.0	310.1	23.0	35.1
A. carbonarius	280.1	272.3	262.1	245.0	25.2	37.6
Cunninghamella echinulata	405.3	392.0	381.3	379.1	18.2	26.0
Mucor racemosus	385.6	360.3	345.1	331.3	26.1	38.6
Rgizopus nigricans	295.0	197.1	290.3	289.6	7.0	10.9

Aspergillus flavus, A. fumigatus and *Fusarium culmorum* appear in the 2-fold igran concentration rather than the other concentrations. On the other hand, *Aspergillus carbonarius, Penicillium chrysogenum, P. lanosum* and *Trichoderma hamatum* appear in the 4-fold igran concentration. *Aspergillus ochraceus* appeared in all igran concentrations, while *Helminthosporium sativum* appears in the recommended and 2-fold doses of igran only.

Species count: *Cunninghamella echinulata* and *Mucor racemosus* were the most abundant species (showing the highest count) in either rhizosphere or surrounding soil of the control plants that was completely eliminated by the presence of igran even at its least applied concentration (Table 3). *Aspergillus carbonareus* and *A. ochraceus* prevailed in both soils in lesser numbers than *C. echinulata* or *M. racemosus* but both persisted in the soil even in the presence of the higher igran levels. The latter organism showed the highest count under the latter igran concentration. These observations indicate that both organisms were highly tolerant to the herbicide. The absence of the most common organisms (*C. echinulata* and *M. racemosus*) as well as the other less common species (*Circinella simplex, Mycelia sterilia, Torula lanosa* and *Trichoderma viride*) allowed the flourishing of *Aspergillus ochraceus.* This new status (in the rhizosphere and surrounding soil) favoured the existence of new fungous species other than those prevailing in the control samples, of which *Fusarium moniliforme, Helminthosporium microsorum* and *Penicillium oxalicum* tolerated the recommended igran dose, whereas *Fusarium solani, Helminthosporium sativum, Humicola bevis* and *Rhizopus nigricans* withstood the double recommended dose. In the meantime, *Acremonium furcatum* and *Trichoderma hamatum* whereas *Cephalosporium acremonium* appeared in the double and 4-fold herbicide concentration and *Srachybotrys atra* in the double recommended dose of the herbicide only.

Soil hydrolytic enzymes: In the absence of igran, soil invertase or amylase activity was far greater in the rhizosphere than the surrounding soil of faba beans whereas the difference in the cellulolytic activity was almost negligible (Table 4). Doubling the recommended dose of igran induced the same effects of the lower dose though the cellulolytic activity in the rhizosphere severely dropped. The largest igran dose application significantly increased the invertase and cellulase activity in the rhizosphere and amylase activity in the surrounding medium without affecting the earlier response of the enzymes to the lower dose application.

Biomass yield: The biomass yield, by the test fungi, was hardly, if at all, affected by the presence of the recommended igran dose; a response that continued with larger dose application except *Aspergillus ochraceus* where it significantly stimulated dry weight gain (Table 5). The latter dose attenuated the biomass gain by the other test fungi except *Rhizopus nigricans* where igran, at this level, still had no effect on dry weight gain.

Discussion

Vicia faba plants tolerate the recommended dose of igran and thus the herbicide was very effective in reducing the fungal, actinomycetal and bacterial count more prominently in the soil, thus causing the maximum increase in the R/S ratio.

It is worth mentioning that several investigators (Amewowor and Madelin, 1991; Rizk, 2001 and others) reported that fungi, bacteria and actinomycetes were more abundant in the root zone of *Vicia faba* than in the surrounding soil. El-Abyad *et al.* (1983) noticed that the count of fungi and bacteria was highly significantly inhibited in the presence of certain biocides during the first week sampling. After 2 weeks there was a gradual build up in soil micro flora that was detected by the significant increase in counts. Taiwo and Oso (1997) isolated *Pseudomonas, Achromobacter, Bacillus* spp, *Aspergillus niger* and *Streptomyces* spp. from the loamy soils treated with some pesticides.

The fluctuations in the fungal populations indicate the very wide range of sensitivity or tolerance of the soil fungi dependent on the various soil contaminants and/or distance from the root exudates (rhizosphere and surrounding soil).

In this connection, it may be mentioned that Heggo (1984) reported that the metabolic activity of the rhizosphere micro flora played an important role in growth of the plant. They may produce degrading enzymes, growth promoting or growth retarding substances for higher as well as lower plants. El-Abyad *et al.* (1983) stated that the addition of biocide, at any concentration, caused a decrease in growth rate of two pathogenic form-species of *Fusarium oxysporum.* All the studied biocides caused inhibition of sporulation and germination of macroconidia of the two organisms. Sawicka *et al.* (1998) studied the influence of imazethapyr and linuron on microorganisms of some legume crops. They showed that both herbicides, stimulated the development of bacteria and Actinomycetes and inhibited the growth of fungi.

The common presence of *Aspergillus ochraceus, Cladosporium herbarum, Cunninghamella echimulata, Mucor racemosus, Penicillium chrysogenum, P. lanosum, Torula lanosa* and *Trichoderma viride* in the rhizosphere and surrounding soil might explain the minor differences in their cellulolytic activity. Whereas the presence of *Alternaria alternata, Aspergillus flavus* in the rhizosphere and *A. clavatus, A. fumigatus, Circinella simplex* and *Mycelia sterilia* only in the surrounding soil might explain the

higher invertase and amylase activity in the rhizosphere than the surrounding soil.

It should be recalled that Tsekova *et al.* (1987) and Korshunov and Loginova (1988) reported that aspergilli were the most active fungous species in the production of amylases. In the meantime, Mitchell *et al.* (1991) demonstrated the release and diffusion of glucoamylase and hydrolysis of starch by *Rhizopus oligosporus*. In the meantime, Kamel (1983) reported that the amylolytic and cellulolytic activities of *Streptomyces* species were higher within the rhizosphere of soybean plants.

The presence of *Alternaria alternata, Aspergillus ochraceus, Cladosporium herbarum, Penicillium lanosum, P. oxalicum* and *Rhizopus nigricans* only in the rhizosphere *and Aspergillus clavatus, Helminthosporium sativum* and *Humicola brevis* only in the surrounding soil of bean plants treated with the recommended igran dose coupled with complete absence of *Aspergillus flavus, A. fumigatus, Circinella simplex, Cunnighamella echinulata, Fusarium culmorum, Mucor racemosus, Mycelia sterilia, Penicillium chrysogenum, Torula lanosa and Trichoderma viride* from either rhizosphere or surrounding soil might explain the drop in invertase activity in both areas. The minor effects on the amylase activity increased the cellulolytic activity in the rhizosphere and insignificantly affected that of the surrounding soil.

Stirban and Soreanu (1975) reported that triazine herbicides inhibited saccharase, dehydrogenase, urease, protease and phosphatase activity of the soil. Hussein *et al.* (1993) controlled the natural infestation of *Orabanche* sp. by glyophosfate application which significantly increased nodulation and nitrogenase enzyme activity of *Vicia faba* plants inoculated with *Bradyrhizobium leguminosarum*.

The biomass yields of the test fungi indicate the differences in the mechanisms of tolerableness to this herbicide. *Aspergillus ochraceus* had the capacity to break igran into simple carbon, nitrogen and sulphur molecules for its own nourishment. In other words, it was able to break the heterocyclic ring into simple nutritive components. *Rhizopus nigricans* was able to block the active and passive pathways of entry of igran through the plasma membrane whereas the remaining test organisms were able of partial breakdown of igran i.e. the side chains of the heterocyclic ring, the latter seemed to be toxic at its higher levels.

It may be recalled that Audus (1976) was of the opinion that stimulation effects in fungi might be due to the use of the herbicides as a nutrient source especially in pure culture. Fenuron stimulated *Aspergillus niger* at the excessive concentration of 1000 ppm when urea was added. Walker (1977) pointed out that microbial breakdown of herbicides fall into one of the two categories: 1- the first group, to which simazine belonged, degradation proceeds at a steady rate approximately proportional to the concentration of the chemicals in the soil, with no lag phase. This was due to microbial enzymes, which degraded natural substrates in the soil. 2- in the second group, slow degradation by the non-specific mechanism, described above, is followed by a period of rapid breakdown. It was thought that microbial enzymes adapt to the new substrate during the initial phase.

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